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# *Brachypodium distachyon tar2l^hypo* mutant shows reduced root developmental response to symbiotic signal but increased arbuscular mycorrhiza

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## ABSTRACT

Auxin is a major phytohormone that controls root development. A role for auxin is also emerging in the control of plant–microbe interactions, including for the establishment of root endosymbiosis between plants and arbuscular mycorrhizal fungi (AMF). Auxin perception is important both for root colonization by AMF and for arbuscule formation. AMF produce symbiotic signals called lipo-chitooligosaccharides (LCOs) that can modify auxin homeostasis and promote lateral root formation (LRF). Since *Brachypodium distachyon* (Brachypodium) has a different auxin sensitivity compared to other plant species, we wondered whether this would interfere with the effect of auxin in arbuscular mycorrhizal (AM) symbiosis. Here we tested whether *tar2l^hypo* a Brachypodium mutant with an increase in endogenous auxin content is affected in LRF stimulation by LCOs and in AM symbiosis. We found that, in contrast to control plants, LCO treatment inhibited LRF of the *tar2l^hypo* mutant. However, the level of AMF colonization and the abundance of arbuscules were increased in *tar2l^hypo* compared to control plants, suggesting that auxin also plays a positive role in both AMF colonization and arbuscule formation in Brachypodium.

Auxin is a phytohormone with a major effect on plant root development.[1] More recently, auxin has been shown to play an important role in the establishment of root endosymbioses such as arbuscular mycorrhizal (AM) or nitrogen-fixing symbioses.[2–4] During AM symbiosis, auxin signaling is activated in root cortical cells in which arbuscular mycorrhizal fungi (AMF) form branched exchange structures called arbuscules. Moreover, exogenous auxin application or downregulation of auxin perception affects arbuscule formation.[3] Since lateral roots (LR) are also preferred sites for AMF colonization,[5] auxin may also affect AM symbiosis through the control of lateral root formation (LRF). The lipo-chitooligosaccharide (LCO) signal molecules produced by rhizobial bacteria[6] and AMF[7] induce LRF in various plant species, including dicots and monocots.[7–10] We recently showed that this probably occurs through modification of auxin homeostasis, leading to an increase of the auxin content in the part of the root containing the LR initiation zone.[9] Whereas exogenous auxin treatment with auxin analogs like Naphthalene-Acetic acid (NAA) increased LRF in most plant species including the monocots rice[11] and maize,[12] it failed to promote LRF in *Brachypodium distachyon* (Brachypodium), and by contrast, tended to inhibit LRF even at low concentrations. Only exogenous treatment with low concentration ($10^{-9}$M) of the auxin precursor Indole-3-Butyric Acid (IBA) promotes LRF in Brachypodium.[9] Similarly, whereas LCOs act synergistically with exogenous auxin for stimulation of LRF in the model legume *Medicago truncatula*,[13] combination of $10^{-9}$M IBA and $10^{-7}$M LCO fails to stimulate LRF in Brachypodium.[9] This suggests that Brachypodium is highly sensitive to exogenous auxin treatment for LRF, probably

because endogenous auxin content is close to the optimal concentration required for LRF. Indeed, Brachypodium shows some specificities for auxin production and accumulation such as a different ethylene-auxin crosstalk wiring controlling auxin biosynthesis gene expression and a different *AUX1* mutant phenotype compared to Arabidopsis.[14,15] These differences in auxin sensitivity and homeostasis compared to other plant species raise the question of the role of auxin during AM symbiosis in Brachypodium.

## Brachypodium roots with higher endogenous auxin content display less lateral roots in response to exogenous auxin or LCO treatments

In order to answer this question, we used *tar2l^hypo*, an hypomorphic mutant of *TAR2L*, a gene involved in auxin biosynthesis in Brachypodium.[15] This mutant shows reduced transcript level of *TAR2L* but a higher endogenous IAA content in primary root as well as a modified root architecture with higher number of LRs.[15] Similarly, in our conditions, *tar2l^hypo* had more LRs than the control in non-treated condition (Figure 1 (a,b)). We tested the effect of IBA or LCO on LRF in the wild type control (Figure 1(a)), plants of the Bd21 genotype regenerated from embryogenic calli. Similarly to what was observed with the Bd21.3 genotype,[9] $10^{-9}$M IBA and $10^{-7}$M LCO significantly increased LRF by 37% and 32%, respectively (from an LR mean number of 13.1 in the control condition to 17.9 or 17.3 upon IBA and LCO treatments, respectively). By contrast, in the *tar2l^hypo* mutant (Figure 1(b)), both $10^{-9}$M IBA and $10^{-7}$M LCO treatments significantly reduced LRF by 31% and 25% (from an LR mean number of 25 in the control condition

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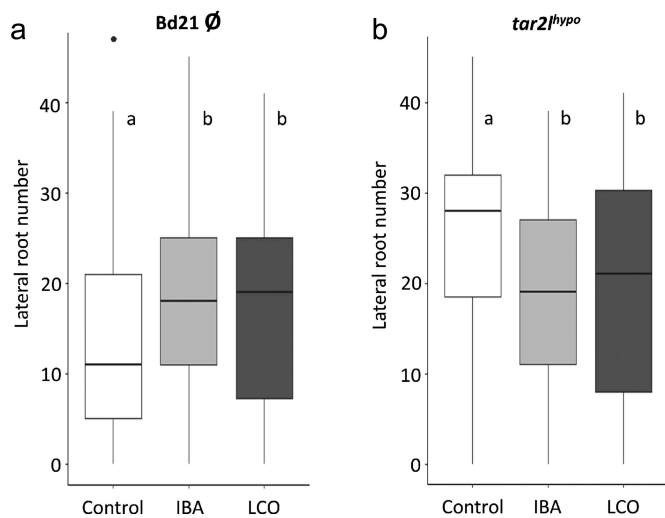
**Figure 1.** IBA and LCO stimulate lateral root formation in *Brachypodium* control plants and inhibit lateral root formation in the auxin overproducer mutant *tar2l^hypo*. Effect of IBA ($10^{-9}$M) and LCO-V (C18:1, NMe, S) ($10^{-7}$M) on *Brachypodium* emerged lateral root number observed at 10-day post-transplantation in wild type control plants (a) or *tar2l^hypo* plants (b) Non-parametric Kruskal–Wallis followed by a post-hoc Van-Werden test was used for statistical analyses. Different letters represent statistically different categories. Data are from three biological replicates (60 to 90 individuals in total).

the endogenous auxin concentration in wild type plants is close to the optimum concentration required to stimulate LRF, and that any higher auxin content inhibits LRF.[9] The negative effect of LCO on the number of LR in the *tar2l^hypo* mutant also fits with the mechanism we suggested whereby LCO application can enhance endogenous auxin level in roots,[9] that might lead to a negative effect on LRF in *tar2l^hypo*.

## Brachypodium roots with higher endogenous auxin content show increased AMF colonization and arbuscule formation

In parallel, we inoculated the *tar2l^hypo* mutant and the wild type control with 200 spores of the AMF species *Rhizophagus irregularis* (DAOM197198, Agronutrition, Carbonne, France), and analyzed fungal colonization by two different methods. We first performed RT-qPCR in order to quantify at 3-week post inoculation (wpi) the amount of AMF in the root system and the amount of arbuscules. We did so by measuring a fungal housekeeping gene (*RiGAPDH*) as in[16] and plant genes whose expressions are highly induced in AMF colonized plants (*Bradi2g51930* and *Bradi2g45520*). *Bradi2g51930* codes for a LysM-domain containing protein (ortholog of the rice *AM3* marker gene induced during AMF colonization[17]) and *Bradi2g45520* codes for a phosphate transporter specifically expressed in arbuscule-containing cells.[18] We observed a significantly higher expression of *RiGAPDH* and *Bradi2g51930* in the mutant (Figure 2(a)) suggesting that

to 17.3 or 18.8 upon IBA and LCO treatments, respectively). These results confirm our initial hypothesis that Brachypodium is highly sensitive to exogenous auxin treatment likely because
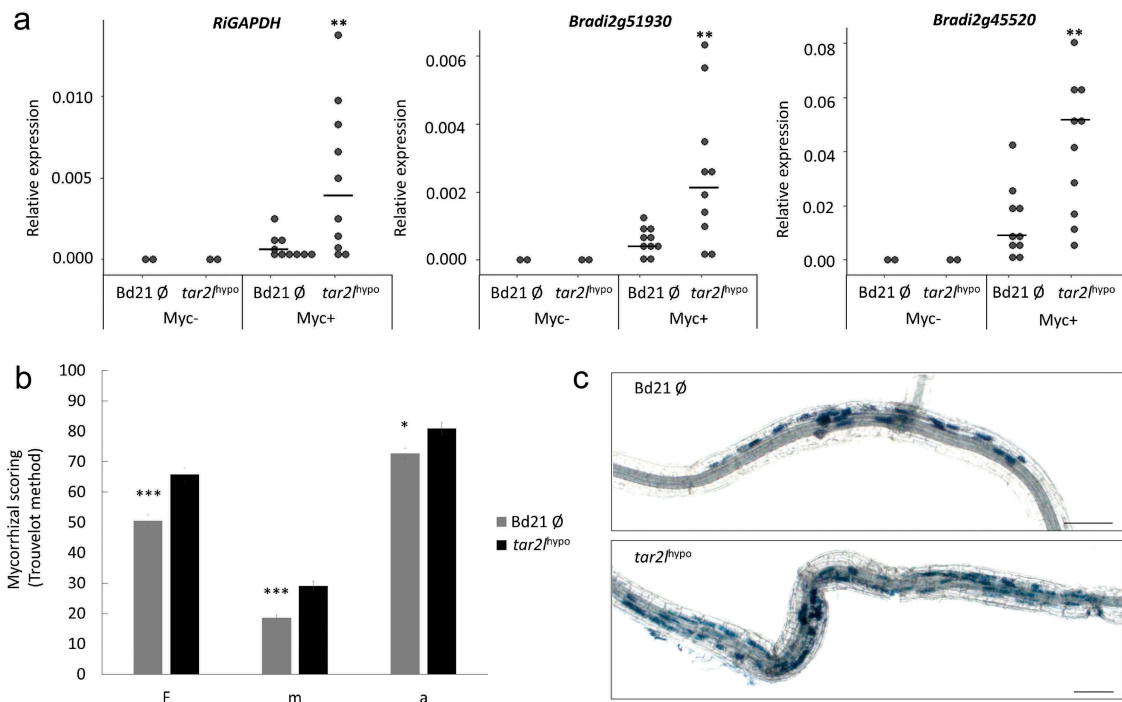


**Figure 2.** The auxin overproducer mutant *tar2l^hypo* is more colonized by AMF than the wild type control. (a) Relative expression of a fungal housekeeping gene (*RiGAPDH*) and plant AM marker genes (*Bradi2g51930* and *Bradi2g45520*) in wild type control plants or *tar2l^hypo* plants, in the absence (Myc-) or presence (Myc+) of AMF. RNA extraction (from entire root systems collected at 3 wpi) and qRT-PCR were performed as described in 16. Expression was normalized to the plant housekeeping gene *BdEF1a* expression. Dot plots show the distribution of 2 to 10 pools of five plants, from 1 (Myc-) or 3 (Myc+) biological replicates. The median is shown for the Myc+ samples. ** = t-test $p < .01$. (b) Frequency of AMF colonization (% of the root system), the intensity of AMF colonization (arbitrary unit, AU) and arbuscule abundance (AU) in wild type control plants and *tar2l^hypo* plants. Eighteen individuals of each genotype were grown in 50 ml tubes filled with attapulgite as described in 16. Thirty root pieces were randomly collected from each plant at 3 wpi, stained and phenotyped according to the mycocalc method [19]. F = frequency of colonization in the root system; m = intensity of colonization in the root fragments; a = arbuscule abundance in the root fragments. Error bars indicate standard error of the mean. * = T-test $p$ value $< .05$; *** = T-test $p$ value $< .001$. (c) Bright field images of control (upper panel) and *tar2l^hypo* (lower panel) roots stained with ink as described in[20] AMF stained in blue. Bars, 100 μm.

*tar2l^hypo* is more colonized than the control. In addition, we observed significantly higher expression of *Bradi2g45520*, suggesting that there were more arbuscules formed in the mutant than in the control. To better understand the effect of the *tar2l^hypo* mutation on AMF colonization, in an independent experiment, we used the mycocalc method[19] to score by microcopy root systems at 3wpi. We observed that *tar2l^hypo* had a significantly higher i) frequency of AMF colonization, ii) AMF colonization intensity and iii) arbuscule abundance (Figure 2(b,c)) compared to the wild type control. Altogether, this shows that the *tar2l^hypo* mutation leads to both an increase in AMF colonization and in arbuscule formation.

This is likely due to the increase in root endogenous auxin content since similar effects were observed after treatments with exogenous auxin or interference with auxin signaling in tomato and *M. truncatula*.[3] Interestingly both AMF colonization and arbuscule formation were increased in the Brachypodium *tar2l^hypo* mutant as in tomato and *M. truncatula* after exogenous auxin treatment while only arbuscule formation was increased after exogenous auxin treatment in rice.[3] This role of auxin on AMF colonization might occur directly by auxin promoting penetration and/or spreading of AMF in roots and/or indirectly by controlling root architecture. Indeed, we cannot rule out that the increased AMF colonization of *tar2l^hypo* roots is linked to the increased LR number or to other developmental phenotypes that might result from the mutation in *TAR2L*. Our results open interesting questions on the possible combined effect of LCOs and auxin on arbuscule formation *in planta*.

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